

575 PCT WO: New claims filed December 2000 - response 1st. written opinion

CLAIMS

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1. A transgenic cereal plant cell comprising a nucleotide sequence encoding a maltogenic alpha-amylase which nucleotide sequence, in the cell, is operably linked to elements required for mediating expression of the nucleotide sequence in the seeds of a cereal plant regenerated from said plant cell, said maltogenic alpha-amylase having an amino acid sequence which has:
 - i) at least 70% identity to SEQ ID NO: 2, preferably at least 75%, 80%, 85% or at least 90%, e.g. at least 95%, 97%, 98%, or at least 99%; or
 - ii) at least 70% identity to the amino acid sequence set forth in amino acids 1-686 of SEQ ID NO:1, preferably at least 75%, 80%, 85% or at least 90%, e.g. at least 95%, 97%, 98%, or at least 99%.
2. The plant cell according to claim 1, wherein the plant cell is a wheat plant cell.
3. The plant cell according to claim 1 or 2, wherein said maltogenic alpha-amylase has the amino acid sequence shown in SEQ ID NO: 2 or the amino acid sequence set forth in amino acids 1-686 of SEQ ID NO:1.
4. The plant cell according to any of the preceding claims, wherein the maltogenic alpha-amylase in baking has an antistaling effect and can be dosed broadly essentially without negative effect.
5. The plant cell according to any of the preceding claims, wherein a seed specific promoter drives the expression of the maltogenic alpha-amylase.
6. The plant cell according to any of the preceding claims, wherein the nucleotide sequence encoding the maltogenic alpha-amylase is derived from a microorganism, preferably a bacterium.

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7. The plant cell according to claim 6, wherein the nucleotide sequence encoding the maltogenic alpha-amylase is derived from the *Bacillus* strain NCIB 11837.

8. A transgenic cereal plant regenerated from a plant cell according to any of the preceding claims and the progeny of said parent plant, which parent or progeny plant is capable of expressing said maltogenic alpha-amylase in the seeds.

9. A transgenic cereal plant comprising a nucleotide sequence encoding a maltogenic alpha-amylase which nucleotide sequence is operably linked to elements required for mediating expression of the nucleotide sequence in the seeds of said plant, wherein said maltogenic alpha-amylase has an amino acid sequence having:

- i) at least 70% identity to SEQ ID NO: 2, preferably at least 75%, 80%, 85% or at least 90%, e.g. at least 95%, 97%, 98%, or at least 99%; or
- ii) at least 70% identity to the amino acid sequence set forth in amino acids 1-586 of SEQ ID NO:1, preferably at least 75%, 80%, 85% or at least 90%, e.g. at least 95%, 97%, 98%, or at least 99%.

10. The plant according to claim 8 or 9 which is wheat.

11. The plant according to any of claims 8-10, wherein said maltogenic amylase is as defined in claims 3 or 4.

12. A seed of a cereal plant defined in any of claims 8-11, said seed containing the maltogenic alpha-amylase in an amount which is effective to delay staling of bread baked from the seed.

13. A transgenic cereal seed comprising maltogenic alpha-amylase in an amount which is effective to delay staling of bread baked from said seed, wherein said maltogenic alpha-amylase has an amino acid sequence having:

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- i) at least 70% identity to SEQ ID NO: 2, preferably at least 75%, 80%, 85% or at least 90%, e.g. at least 95%, 97%, 98%, or at least 99%; or
- ii) at least 70% identity to the amino acid sequence set forth in amino acids 1-686 of SEQ ID NO:1, preferably at least 75%, 80%, 85% or at least 90%, e.g. at least 95%, 97%, 98%, or at least 99%.

14. The seed of claim 12 or 13, wherein said maltogenic alpha-amylase is as defined in any of claims 3 or 4.

15. The seed of any of claims 12-14, wherein said seed is wheat.

16. Use of seeds as defined in any of claims 12-15 for the preparation of flour or dough.

17. Use of seeds as defined in any of claims 12-15 in baking.

18. A method for preparing a baked product, said method comprising the steps of:

- i) expressing a maltogenic alpha-amylase in the seeds of a transgenic cereal plant, said maltogenic alpha-amylase having an amino acid sequence which has:
 - a) at least 70% identity to SEQ ID NO: 2, preferably at least 75%, 80%, 85% or at least 90%, e.g. at least 95%, 97%, 98%, or at least 99%; or
 - b) at least 70% identity to the amino acid sequence set forth in amino acids 1-686 of SEQ ID NO:1, preferably at least 75%, 80%, 85% or at least 90%, e.g. at least 95%, 97%, 98%, or at least 99%;
- ii) preparing flour from said cereal seed comprising said maltogenic alpha-amylase;
- iii) preparing a dough comprising the flour of step ii); and
- iv) baking the dough to obtain a baked product.

19. A method for preparing a baked product, said method comprising the steps of:

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i) preparing flour from cereal seed, said seed comprising a maltogenic alpha-amylase having an amino acid sequence which has:

- a) at least 70% identity to SEQ ID NO: 2, preferably at least 75%, 80%, 85% or at least 90%, e.g. at least 95%, 97%, 98%, or at least 99%; or
- b) at least 70% identity to the amino acid sequence set forth in amino acids 1-686 of SEQ ID NO:1, preferably at least 75%, 80%, 85% or at least 90%, e.g. at least 95%, 97%, 98%, or at least 99%;

ii) preparing a dough comprising the flour of step i); and

iii) baking the dough to obtain a baked product.

20. A method for preparing a baked product, said method comprising the steps of:

i) preparing a dough from flour obtained from cereal seed, said seed comprising a maltogenic alpha-amylase having an amino acid sequence which has:

- a) at least 70% identity to SEQ ID NO: 2, preferably at least 75%, 80%, 85% or at least 90%, e.g. at least 95%, 97%, 98%, or at least 99%; or
- b) at least 70% identity to the amino acid sequence set forth in amino acids 1-686 of SEQ ID NO:1, preferably at least 75%, 80%, 85% or at least 90%, e.g. at least 95%, 97%, 98%, or at least 99%;

ii) preparing a dough comprising the flour of step i); and

iii) baking the dough to obtain a baked product.

21. The method according to any of claims 18-20, wherein the maltogenic alpha-amylase is as defined in claim 2 or 3.

22. The method according to any of claims 18-20, wherein said seed is as defined in any of claims 12-15.